An1cca's notes on cactus phytochemistry

September 21, 2017

Dear reader,

By providing experimental techniques and results concerning the detection, quantification and optimal extraction of the cactus alkaloid M, my hope is that more users can become self-sufficient in their need for entheogenic catalysts. That way, the natural populations and habitats of these sacred cacti can be left undisturbed and their entheogenic use becomes sustainable. It is my belief that the consumption of these entheogens should always be the result of an informed and free choice and that the experience that it delivers should be honored and enjoyed within a safe and well-prepared context. I also believe that having access to entheogens of reliable purity brings a sense of trust to the experience, especially in difficult moments.

By no means do I advocate the use of these plants or substances. Although their use seems to be beneficial for many, this may not be the case for you. So please inform yourself thoroughly before deciding to embark on these inner journeys.

This document first describes the process of DCMQ (Direct Chromatographic M-Quantification) using TLC. In a second part, I discuss extraction and purification techniques that transform a fresh cactus cutting to crystalline M-SO4. I hope these notes may help other researchers take the observations and prectices as a start for further experiments so that they may be confirmed, rejected and expanded upon. That's why I upload this document to the Nexus, which I think is a fertile soil for the further growth of entheogenic knowledge.

I wish you safe and inspiring journeys!

Cheers ☺,

An1cca

Direct Chromatographic M-Quantification

DCMQ = ‘Direct’ because there is no drying/extraction step involved. Even no harvesting! ‘Chromatographic’ because TLC is used as the combined extraction, separation and visualisation-method. ‘M’-Quantification: speaks for itself, or is it the sugar-content you were looking for ☺?

What are possible uses for this technique? It seems that it can differentiate between weak, medium and strong M-producers in a quick, non-harmful and (semi-)quantitative manner. It could be used to screen specimens in large nurseries, or even in the wild. Originally, I developed the technique to screen seedlings for their M-production, so that very early in the growing process, one could select these out. As we will see however, small cacti up to 5cm do not seem to produce M in detectable quantities ☹.

Another use for DCMQ would be to estimate the strength of a cutting before consumption. Too often I see reports of disappointed (and nauseous!) psychonauts who have done their best effort to prepare a cactus that contained hardly any M. Having had such an experience, another meeting with this plant-teacher becomes quite improbable. For psychonauts interested in consuming ‘full -alkaloid extracts’, TLC-comparison with a known reference will tell them how many M they will be consuming. Just because they don’t have to drink the bitter snot-teas anymore, doesn’t mean they don’t prepare and make time for this 12 hour experience. And if they’re anything like me, they will like to know how much they’re taking of what.

Yet another use for the technique may be the testing of various means of raising the M-content, because this kind of sampling allows the individual to live on, inflicting only minimal damage (which could indeed be interpreted as ‘stress’ as well). So we could test for optimum harvest time, influence of watering,… For now, I’m testing the possible rise in M-content by letting the cuttings lie in darkness for many weeks. One thread spoke of a doubling of M-concentration. Well it should be within this technique’s capabilities to show this to be true or not. What I can say already on this is that I tested a bridgesoid-cutting after 6 weeks of which I had samples frozen at harvest. The M-content did not seem to have gotten any higher ☹, in spite of the cutting drying out. I’ll check again in another 6 weeks. Later results from pachanoi cuttings seem to point in the direction of a modest increase (see below).

The technique has now been put to use on Trichocereus, but can most likely be used on Lophophora as well. I think some more dilution of the samples will be necessary however. This might help in selecting strong hybrids, so that cultivation becomes more worthwhile at a time when natural peyote habitats are plundered.

More uses will probably come up along the way when people start experimenting. I’m already looking forward to hearing from them on the Nexus!

In its simplest form, a biopt is taken, put in a small recipient, is then homogenized and spotted on a TLC plate next to a known reference. This procedure is easy and effective. However, when I came to test a non-bridgesoid, a typical peruvianus clone more precisely, the sliminess of the raw material made it impossible to load it into the glass capillaries used for spotting. So I tried diluting the juice. No success. I even bought enzymes that would degrade the responsible molecules. Clearly not the correct ones. Eventually, I remembered how the sliminess of cactus-tea dissappeared after having been boiled for a long time. So I introduced a pressure-cooking step. 1 hour at 120°C finally did the trick! And since I wanted to compare strenghts between different species as well, I added this step to the protocol, even for non-slimy clones.

An important question was how to standardize a sampling procedure when dealing with such small amounts. Scales that are precise enough for this job cost a lot of money and the amount of green epidermis vs the rest is crucial giving its much larger alkaloid content. So the sampling technique had to be standardized as well. That's where the punch biopsy came in. This device is able to take a cylindrical sample of exact and consistent proportions from each different individual. I weighed these samples and came to the conclusion that the variation was quite small (more than 90% weighed between 42-48mg). Even then, the difference in weight could be explained for the most part by a difference in white tissue, due to the technique of 'wiggling' the sample loose within the cactus. If I corrected my results to account for these differences in weight and treated the sample as uniform, the results falsely predicted a higher content (4-6% deviation or more in cases where the sample weighed 34mg). This in fact questions the weighing that is done in some of the following protocol-steps and pleads for a simple punch, dilute and homogenise procedure. But again, I invite everyone to do further research so that we may know for sure.

Before trying the raw, 'Direct'-spotting of sample, I tried some micro-extraction techniques with methanol/NH4OH but these were laborious and difficult to standardize. This is however how I came to the idea of doing the extraction and elution with the same solvent and later on, to do the extraction and elution at the same time by this single solvent on the plate itself. Can you imagine the thrill I got when I saw my first M-spots of raw cactus-juice?

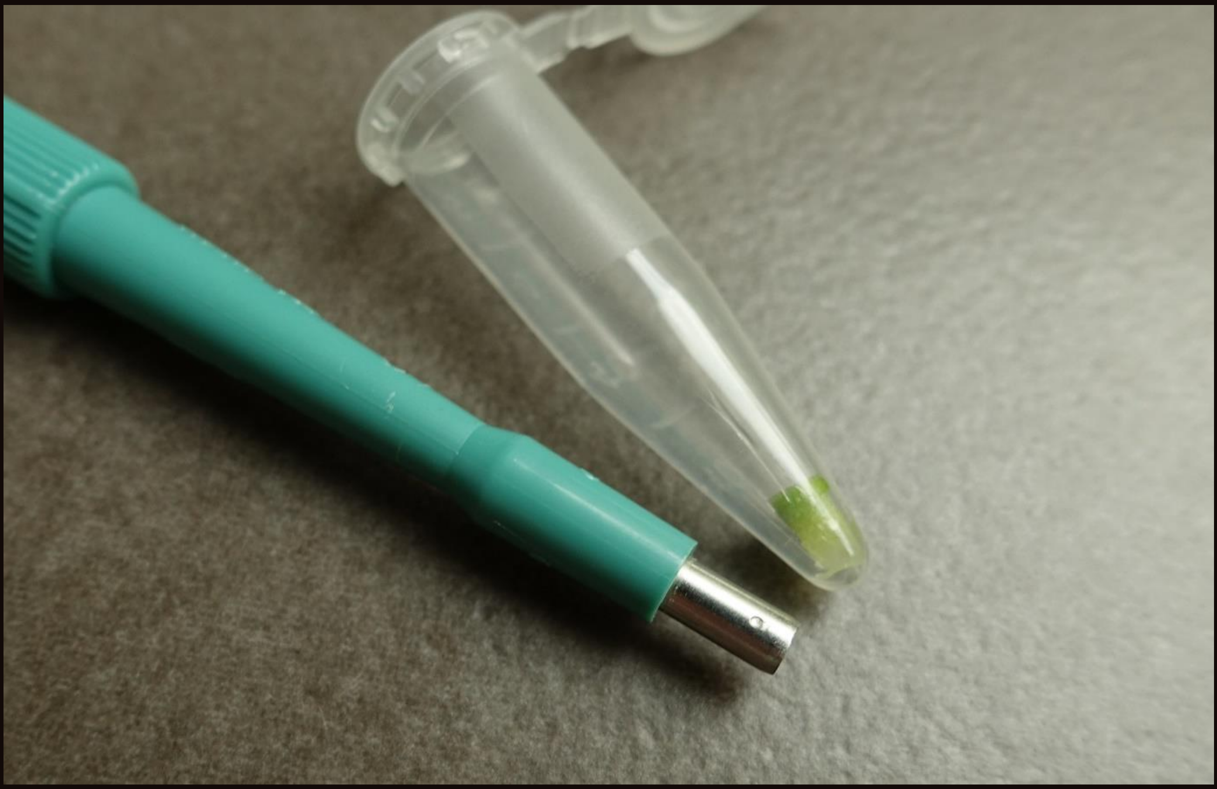
I bought my TLC-kit from Bunk Police and I must say it is very complete. They even supply a handy UV-C light to screen your plates. So this is what I used first to semi-quantify my TLC-spots. However, when working with raw juice, there is a lot of junk that is pulled along the plate and this really makes it hard to visualize and analyze the localized M-spots. So I did my homework and came to know that ninhydrin could selectively visualize amines/alkaloids. After some experimentation and standardization, a working protocol was obtained.

A crucial element in this whole procedure is the final quantification on the basis of spot size. There is an excellent free program that allows one to open a standard picture of the plate and after a few clicks, gives you semi-quantification of the spots: 'ImageJ', or 'Fiji'. All credits go to these programmers, nice work!

I finetuned the technique while selecting the best clones from 41 grown-up individuals that a friend of mine has growing in his greenhouse. Wanting to know which ones he should keep for propagation and which ones he could give away, the technique was put to the test by extracting some individuals and relating the recrystallized amount of M-SO4 with the chromatographic readings. This is what I have done now on 5 clones. The results are listed at the end of the protocol and will hopefully be expanded in the future.

So here we go:

1) Taking the sample: for this, the dermatologists devised an interesting instrument: a circular blade that is attached to a pencil-like handle. Diameters range from 1mm to 8mm. What the most ideal size is, can be determined in subsequent research, but because I wanted to apply the technique to very small cactusses, I preferred a 3mm blade. As it turned out, larger diameters tend to pose problems trying to tear the sample loose on the inside. So it's better to take multiple 3mm samples. To take the sample, the blade is rotated down the skin of the cactus while applying slight pressure. I prefer to take the samples on the flat sides of the ribs to prevent the notch in the middle to keep the blade from going all the way down. I also choose a spot halfway between the areoles, to prevent contamination with fibrous tissue that surrounds the areoles. After it is inserted, the bottom of the punch that is inside the cactus is wiggled in all 4 directions. Then, your thumb is placed on the open end of the punch and it is withdrawn from the cactus. If all went well, you should now see the bottom part of sample that is level with the blade. It seems that it is not a big problem if your sample is somewhat larger or smaller, for it is the green flesh that for the largest part determines the predictions, and even in the fattest specimens I sampled (circumference 22,5cm), the epidermis was no more than 2,5mm thick. Then, the sample is pushed out of the punch blade into a small vial. I ordered some 1,5ml 'Eppendorf' or PCR-vials that are readily available and cheap. A number of them are included in Bunk Police's kit as well.



Now, I weighed all the sample vials beforehand so that I could know the weight of the sample and how much water to add (later on). This however does not seem to be necessary and leaving out the weighing in all of the steps really makes the process easy-going. To be confirmed!

2) Now the samples are frozen for at least 8 hours. This allows one to spread the analyses in time, allows one to compare samples from earlier moments in the specimen's life (right now I can already say that M-content does not significantly degrade over the course of 2 months at -18°C), and will make the homogenisation step easier by disrupting the cells (think of frozen lettuce).

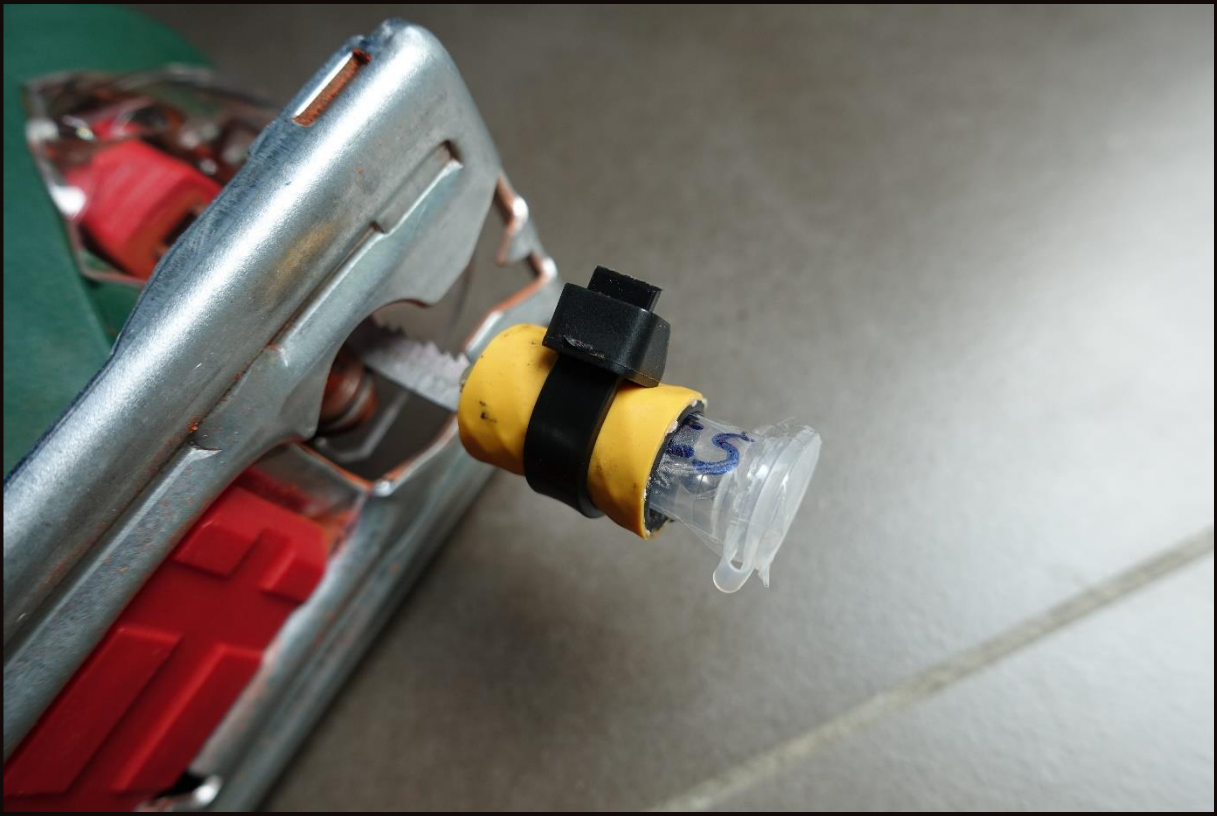
3) Next, the vials are taken out of the freezer, are brought to room temperature and a few (10) small drops of demineralized water from an insulin-syringe are added. These amount to roughly 30 microliter. The idea here is to allow the sample to be heated in a little water to aid in extraction. This is how I have done it up until now, for I had the exact weights of the vials and could add water until the vial was 100mg heavier than its empty weight. However, if indeed we could skip on all the weighing, then we could now just add a fixed amount of water. From experimentation, I would suggest adding 50 microliters of water to a sample of 3 by 6 mm that weighs approximately 45mg. This will be enough dilution to allow the 'soup' to be sucked up in a capillary, while still being concentrated enough to allow for good visualization later on. The samples are then covered with a piece of aluminium-foil that is also put over the label that you put in on the tube. The samples are kept upright in a beaker of some sort and the beaker itself is loosely covered with a sheath of aluminium foil. This assembly is then put into a pressure cooker that was preheated to boiling. The cooker is closed and the samples are autoclaved for 1 hour at 120°C after which they are removed and allowed to cool. I did a lot of weighing on samples before and after cooking, and was surprised to see that practically no liquid cooked out of the vials. I think this might be because when the pot is already warmed up, the pressure buildup all around the samples is very quick so that they are not allowed to come to a boil.

4) If you weighed the vials beforehand and added an imprecise amount of water of roughly 30 microliters in the previous step, now comes the time to weigh each separate vial and add just enough water so that the net weight is 100mg more than the empty weight. If you added a known amount of water, just continue.

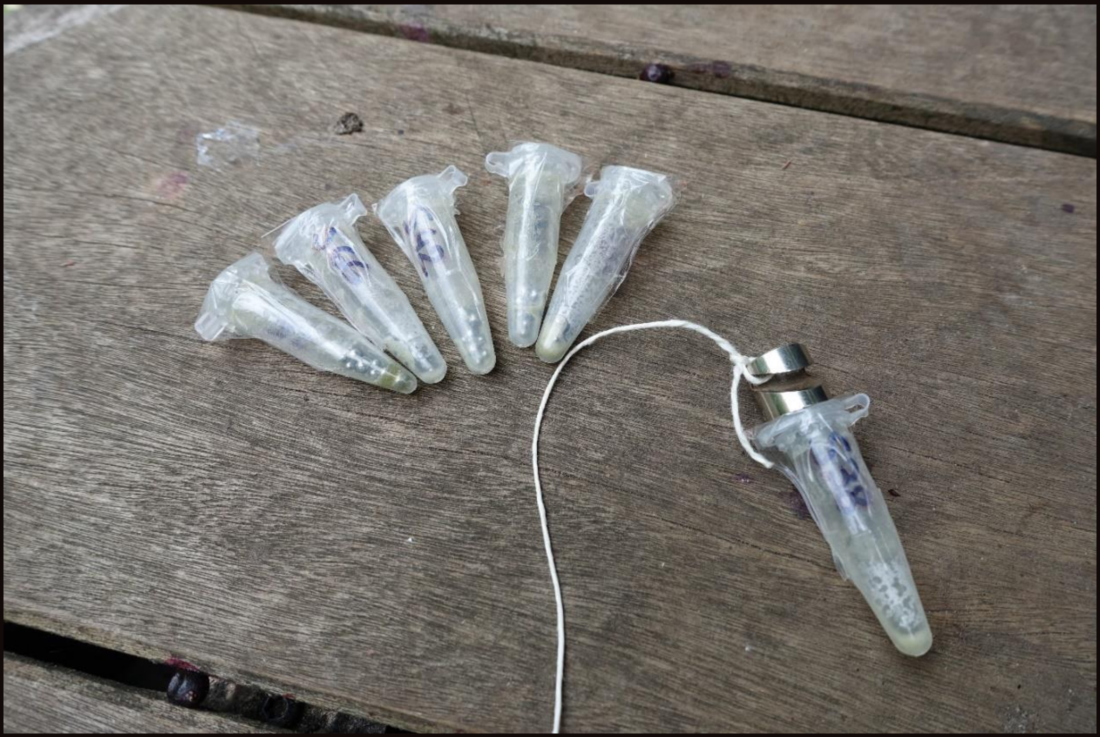
As you can see, I have scale that is only precise to 2mg. However, I prefer to use this one for it gives very repeatable results. I also worked with a Kern ‘Tab’ that weighs precise to 1mg, but the weighings were not repeatable. Kern also sells a single-cell analytical balance for about 700 euro’s that is precise to 0,1mg. It’s nice to have something to dream about 😊. Just to say that I have quite a big measuring error while weighing (+/- 2mg / 100mg, 3 times). So also in this regard, a validation of the no-weighing DCMP-protocol would make it accessible for every grower, whether or not he/she has a precise scale.

5) Homogenization. Here comes a 500 dollar tip: use a jigsaw to make your own ‘bead-beater’! Order some 2 and 3mm steel balls (ball-bearing (online) shops,…) and put 4 balls of each in each vial.

Then, secure the lid by taping it over with clear tape. Now attach the vial to a jigsaw-blade (I used a piece of waterhose tubing that I tie-wrapped around a blade), set it to ‘pendulum’ mode if available and give it a hell of a shake!

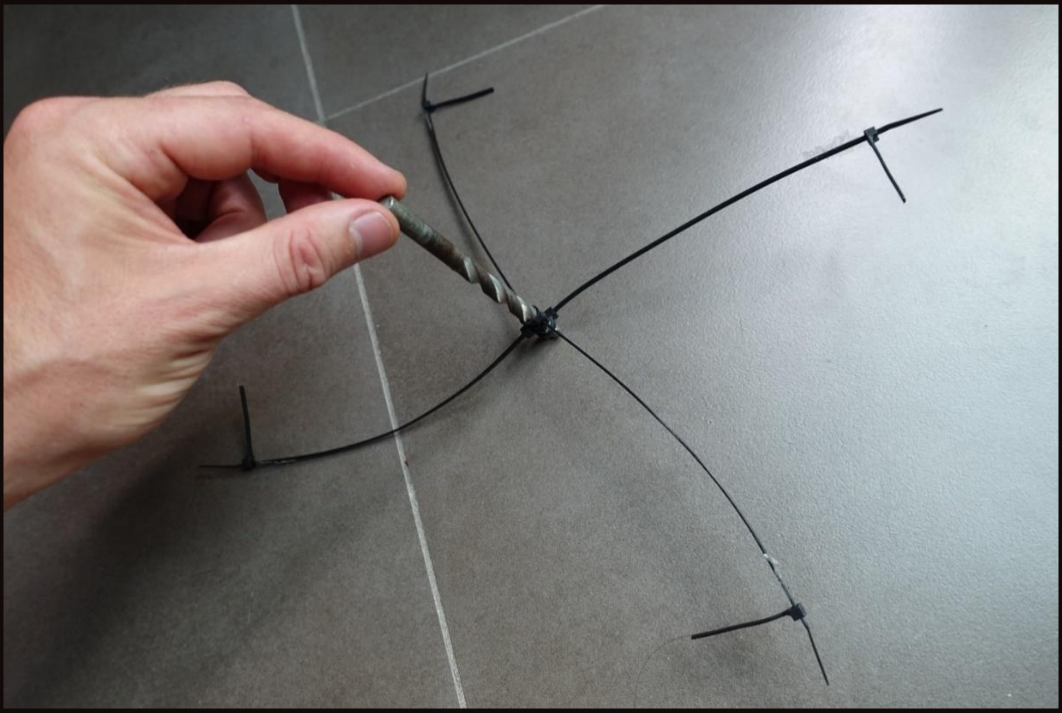


It’s best to use a jigsaw with variable speed here, I cracked a few vials using one that only has an on/off switch. After only 60 seconds of shaking, you will see that your cactus-soup has turned into a frothing foam that coats the vial and steel balls. Now, I attach a neodymium-magnet to a small string of about 50cms lenght and use it to ‘pick up’ the balls and move them up inside the lid. Then swing the vial around for half a minute.

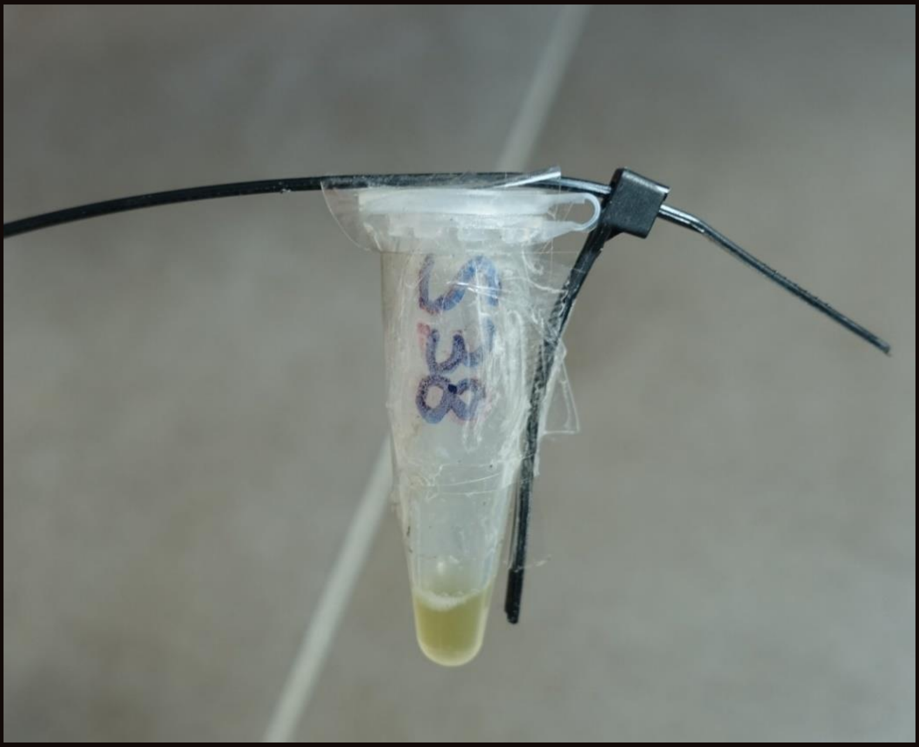


This effectively centrifuges the froth down and turns it into a homogenous solution. Now the tape is cut with a sharp knife and the lid is opened, emptying the balls into a sieve for rinsing and drying (they rust quickly!).

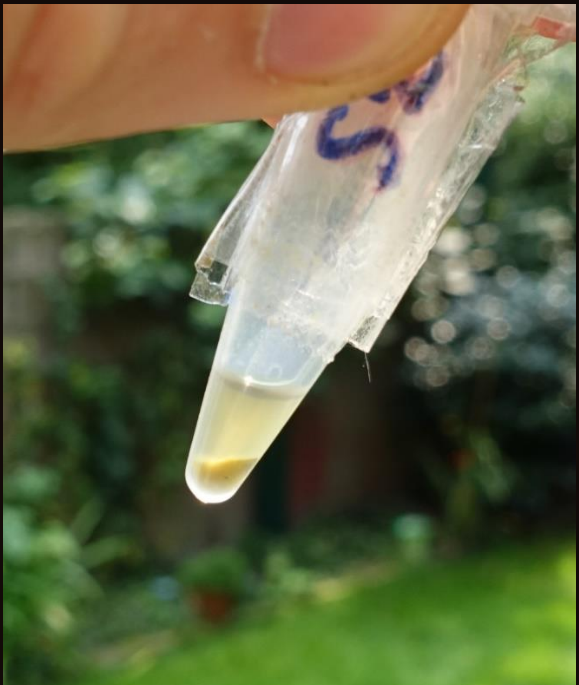
6) Centrifugation: another 500 dollar tip: use your drill as a centrifuge! A number of tie-wraps are attached to a drill bit and at the open end, another tie-wrap is locked and cut off at about 5 cm length.



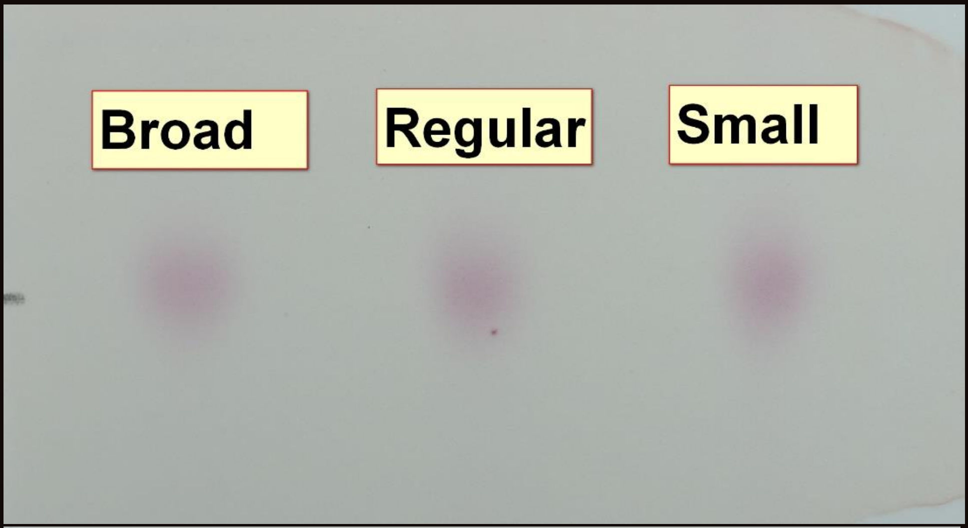
7) Then a vial is securely taped to the first tie-wrap as well as to the second.

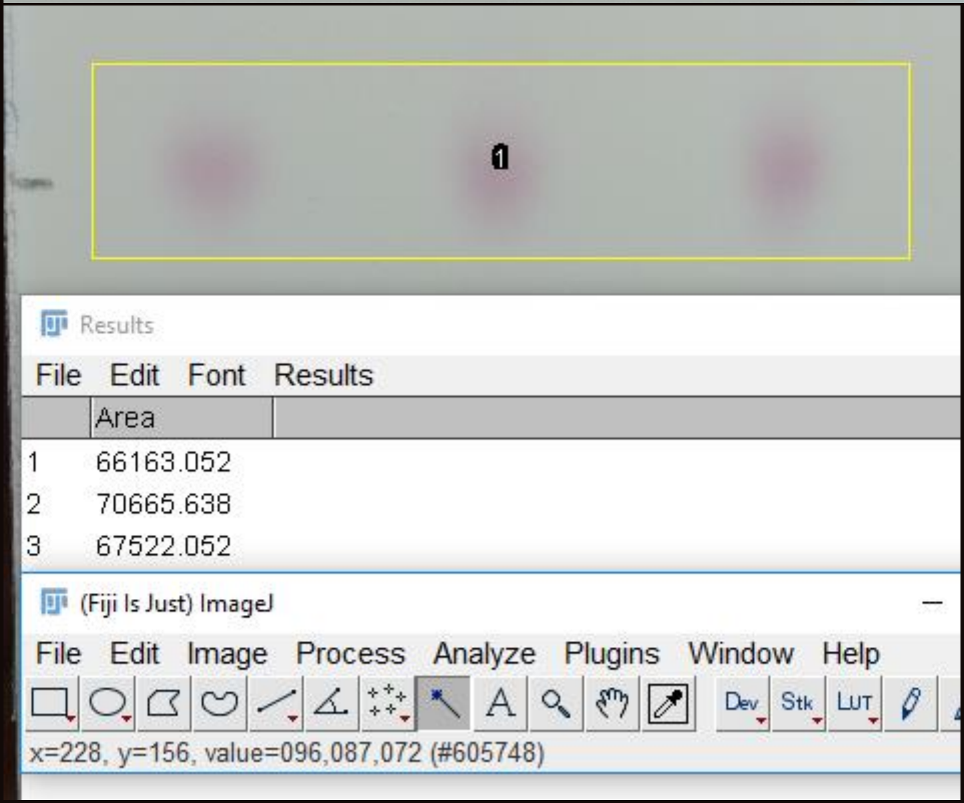


Now the drill is spun inside a large bucket or cardboard box, eye protection is recommended. The speed is gradually increased, and the samples are spun at maximum speed for a minute. This is enough to pack the loose tissue into a compact ‘pellet’ at the bottom of the vial. With a sharp knife, the tape over the lid is cut after which the vial can be pulled loose from the second tie-wrap. This step could be omitted, but in my experience, the plant debris that floats in the solution keeps the capillaries from filling quickly and completely. Besides that, in my TLC experimentation, end results are comparable/identical.



8) Spotting the sample. In Bunk Police’s kit, a number of glass capillaries are provided that have a capacity of somewhat more than 10µl. For reasons of precision, I bought 6,66µl capillaries that are the same length. Measuring errors being smaller this way when wanting to take ½ or ¼ of a capillary. Moreover, I have found that a single 6,66µl capillary completely filled is a fine volume for spotting and visualization. This makes them easy and exact to use.
Spot size isn’t all that critical. I did a test plate with different spot sizes from extra small 3mm (lots of waiting) to extra large 6mm, spotting in 2 times. During all spotting, I set up a heat-gun at 50°C to blow over the plate while spotting. This considerably speeds things up. There were no critical differences observed between the spot intensities.





	Area
1	66163.052
2	70665.638
3	67522.052

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x=228, y=156, value=096,087,072 (#605748)
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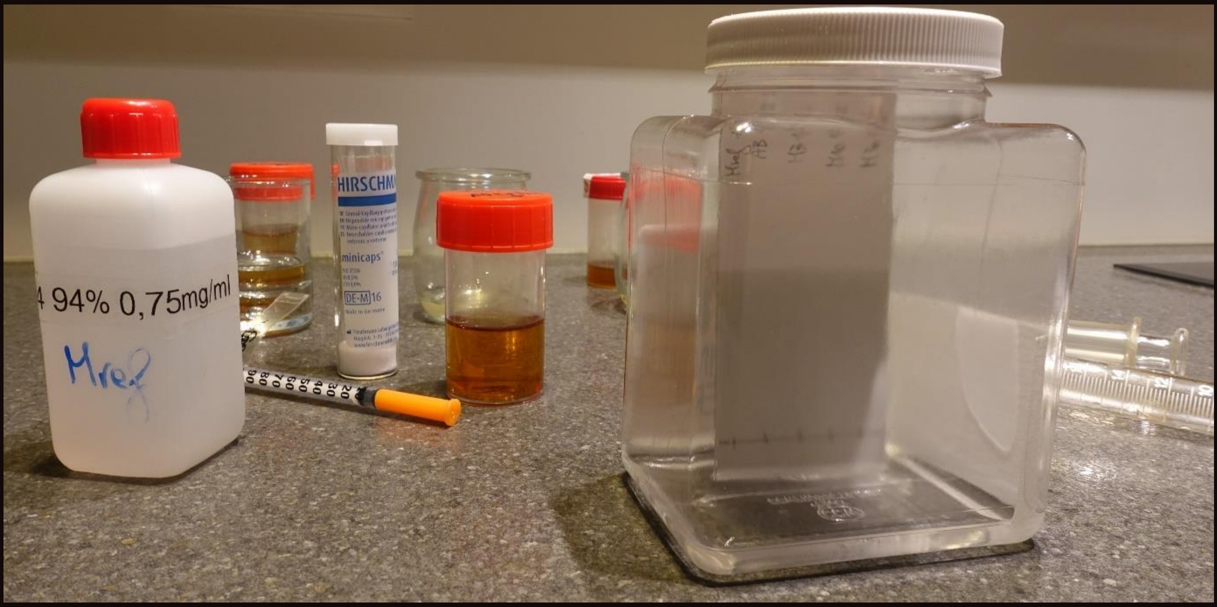
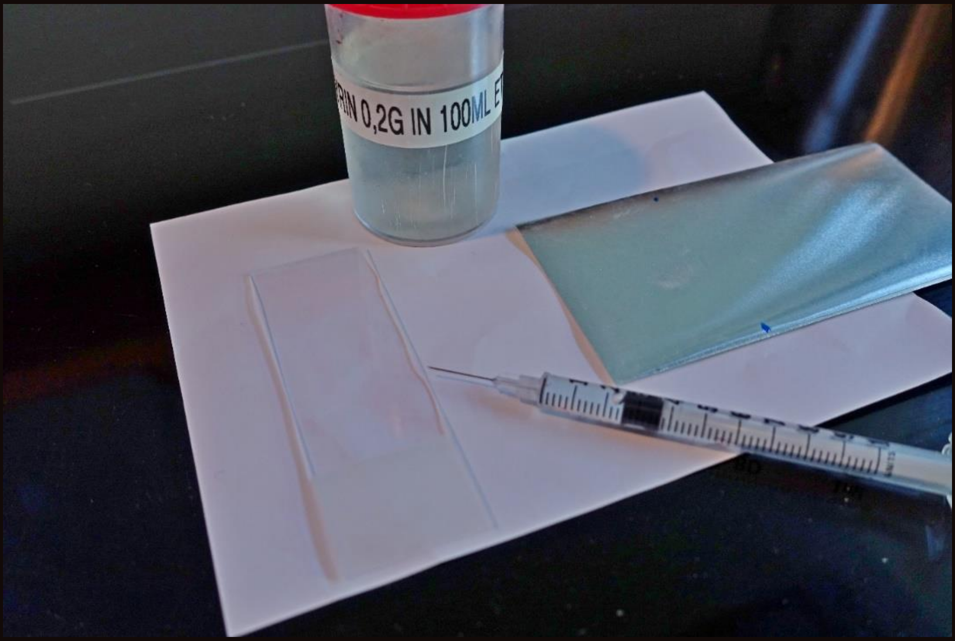
A little word concerning the reference spot. I use a solution of M-SO4 94% 0,75mg/ml as a reference standard. It was made using recrystallized M from previous extractions and was tested by Energy Control (thanks Endlessness!) to confirm its purity of 94%. From my experimentation, it was observed that 3,33µl of this 0,75mg/ml solution was the right amount for comparison of cactus-tissue within the M-range of 0.1-1+%. This equals 2,5µg of M-SO4 per spot. If you spot more, the intensity of the spot will ‘overshoot’ the ‘comparison range’ when using Fiji. Later on I diluted the standard to half its strength so that I could completely fill a 6,66µl capillary and thus eliminate measuring errors.

9) After spotting, the plates are eluted with Bunk Police’s eluens. I think it’s 100/2 methanol/ammonia (24,5%). This gives M a retention factor Rf of about 0,25. It takes about 18 minutes to develop a plate. Below is a picture of the TLC-process while testing crude extracts for their M-content. At the left you see the reference standard . In Bunk Police’s ‘developing chamber’, one can see the eluent is halfway the plate. Insulin syringes are cheap and precise, although its’s better not to use them when working with solvents like acetone.



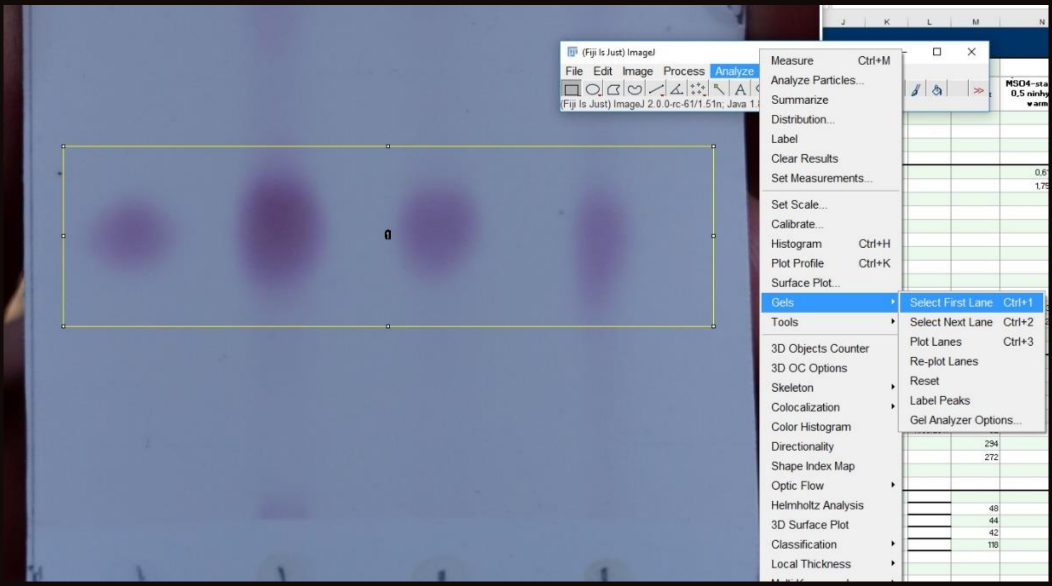
After development, the plates are thoroughly dried outside or in a well-ventilated area.

10) Next comes the staining-step. As I have already mentioned, this step selectively colors amines and is essential to quantifying spot-intensity on TLC because of the UV-blocking smear that crude matrials leaves on the plate. UV-C visualization can however be used when using more purified material (crude extracts). I started off using a ninhydrin solution of 0,2g in 100ml ethanol and spraying it onto the plate. This technique however, is very lossy and very messy (if the stuff touches your skin it will stain it purple for the next 24 hours). So I experimented until I found an alternative method that works very well. The alcoholic ninhydrin-solution is layed out in a flat puddle onto a microscopic slide. Then the position of the M-front is indicated on the back of the TLC-plate. Now the plate is pressed onto the slide making sure that the M-front is located in the middle. When using the right amount of solution, it gets all sucked up in the plate in the course of seconds after which the plate is taken off the slide and set aside. I use about 0,25-0,3 ml of ninhydrin solution. The amount varies according to the thickness of the silica layer. Bunk Police’s plates suck up 0,3ml.

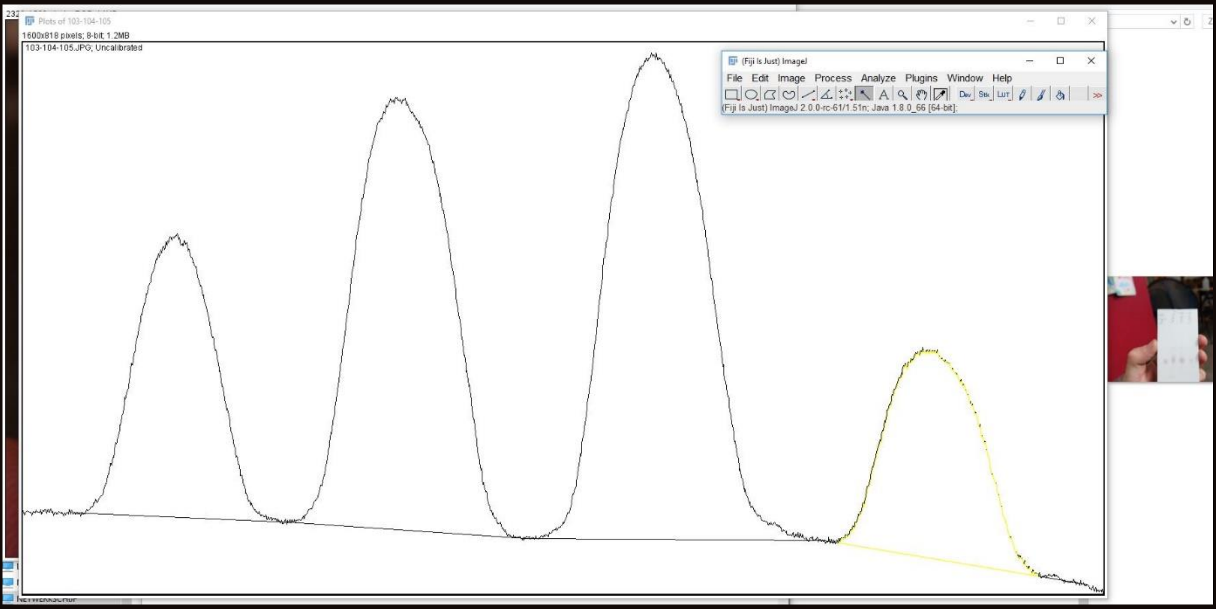


11) Ninhydrin takes a little time to react with the present amines. The warmer, the quicker. Now, just before you decide to put the plate in the oven at 120°C for 15 minutes (which will certainly do the trick), I will tell you that there's an optimum of staining that allows complete comparison whithout 'blowing out' the color intensities of the background or too concentrated extracts. After some experimentation, this optimum was found to be at the point where the streaks above and under the M-spots just become visible. You can choose to just let your plate sit for about 20-30 minutes, or heat it gently with a blowgun until the streaks become just barely visible. At this moment, a well-lit picture is taken. Contrary to an article describing an analogous technique using software named 'TLC-analyzer', the precise lighting conditions do not need to standardized when using a reference spot. However, more contrast makes the curves in Fiji more defined. So I process each picture by cutting out the strip of spots, and increasing the contrast a little (+/-15%). Then I open the picture with Fiji: http://imagej.net/Fiji/Downloads.

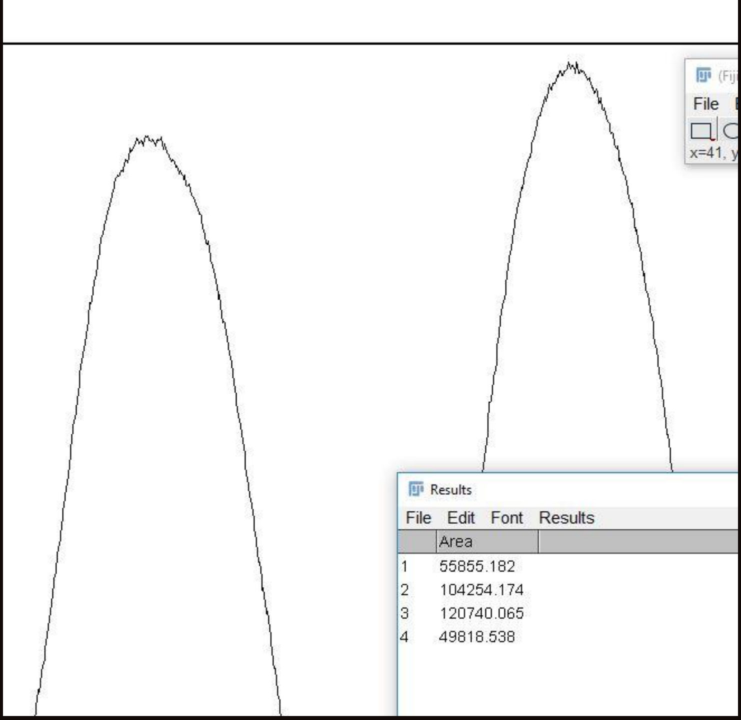
12) First you start by selecting the lane of M-spots.



Then you go to ‘analyze’, ‘gels’, ‘select first lane’. You confirm that the lane is horizontal and then select the ‘plot lanes’ command in the same submenu. The picture above is made at the optimal moment where surrounding streaks become just barely visible.


Plots of 103-104-105
1600x818 pixels; 8-bit; 1.2MB
103-104-105.JPG; Uncalibrated
(Fiji Is Just) ImageJ
File
Edit
Image
Process
Analyze
Plugins
Window
Help
Dev
Stk
LUT
(Fiji Is Just) ImageJ 2.0.0-rc-61/1.51n; Java 1.8.0_66 [64-bit];

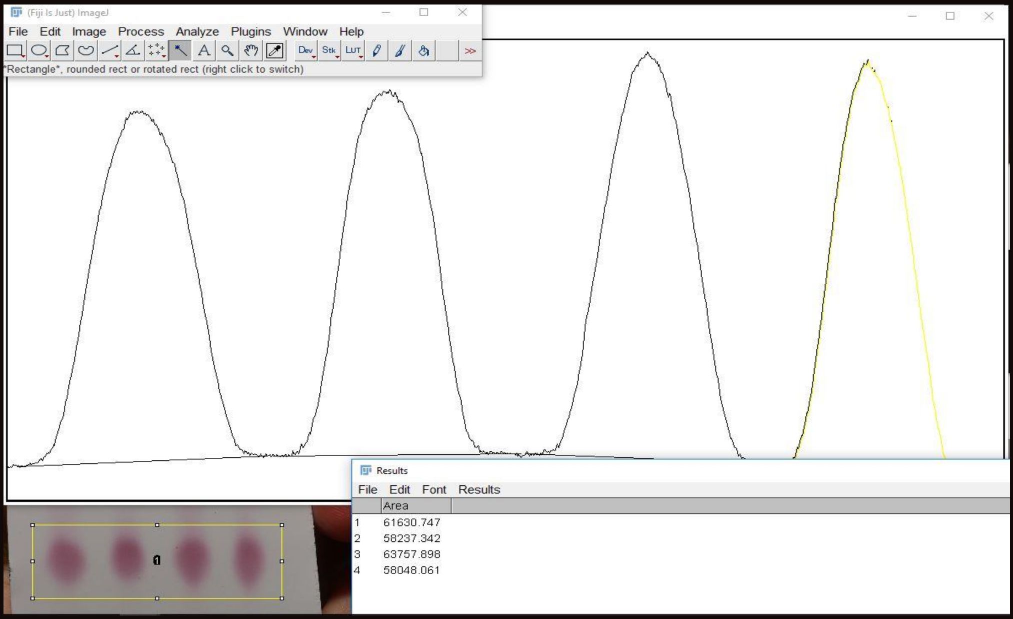
Note that the lanes plotted above were from another plate, sorry for that. On the ‘plot lanes’-screen, draw lines just under the peaks. Then select the ‘magic wand’ tool from the control box and select each peak once. With every click, the surface of the peak is calculated and recorded in a ‘results’-screen.



	Area
1	55855.182
2	104254.174
3	120740.065
4	49818.538

I always make the left spot my reference spot. The relative values of the other spots are easily calculated.

Below is an example from an experiment I did tot determine if pressure-cooking would degrade M-content. Apparently, the jury is still out. Lanes 1&3 are spotted from a fresh biopsy that was diluted 50/50, homogenized and centrifuged. After spotting, the sample was autoclaved vor 1 hour at 120°C. From the resulting solution, lanes 2&4 were spotted. Notice the reproducibility of the method if carried out in the manner outlined above.



"Rectangle", rounded rect or rotated rect (right click to switch)

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   Area
1  61630.747
2  58237.342
3  63757.898
4  58048.061
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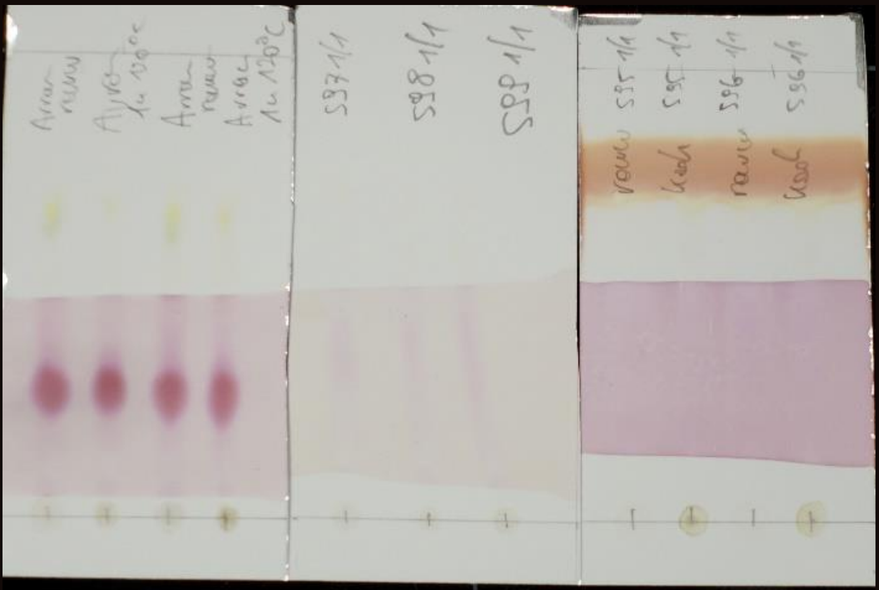
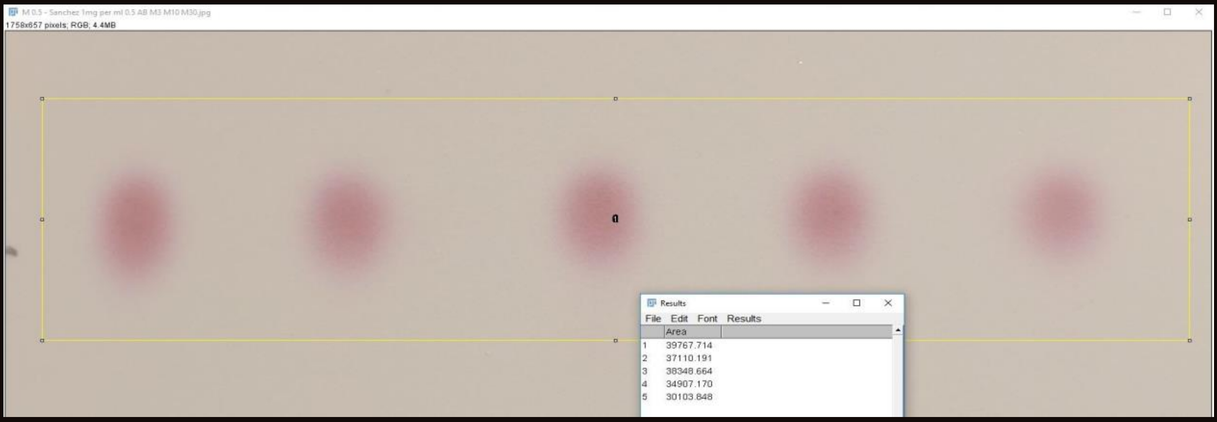
Another example below where the plate mentioned above is put next to two other plates that were spotted with *undiluted* fresh/cooked, homogenized and centrifuged slices of seedlings about 5cm tall. Unfortunately, no M at all could be visualized. The seedlings nevertheless have very promising genetics (Misplant's huaranzensis x scopulicola, huarazensis x lumberjack, huarazensis x psychonaut and lumberjack x psychonaut). So it seems it's impossible to select the small cacti at this stage of development. I might post the follow-up results in the coming years or will gladly hear it from you 😊.



As you can see from the right plate, after a while the ninhydrin stains the background as well, the reason for photographing the plate at the right moment.

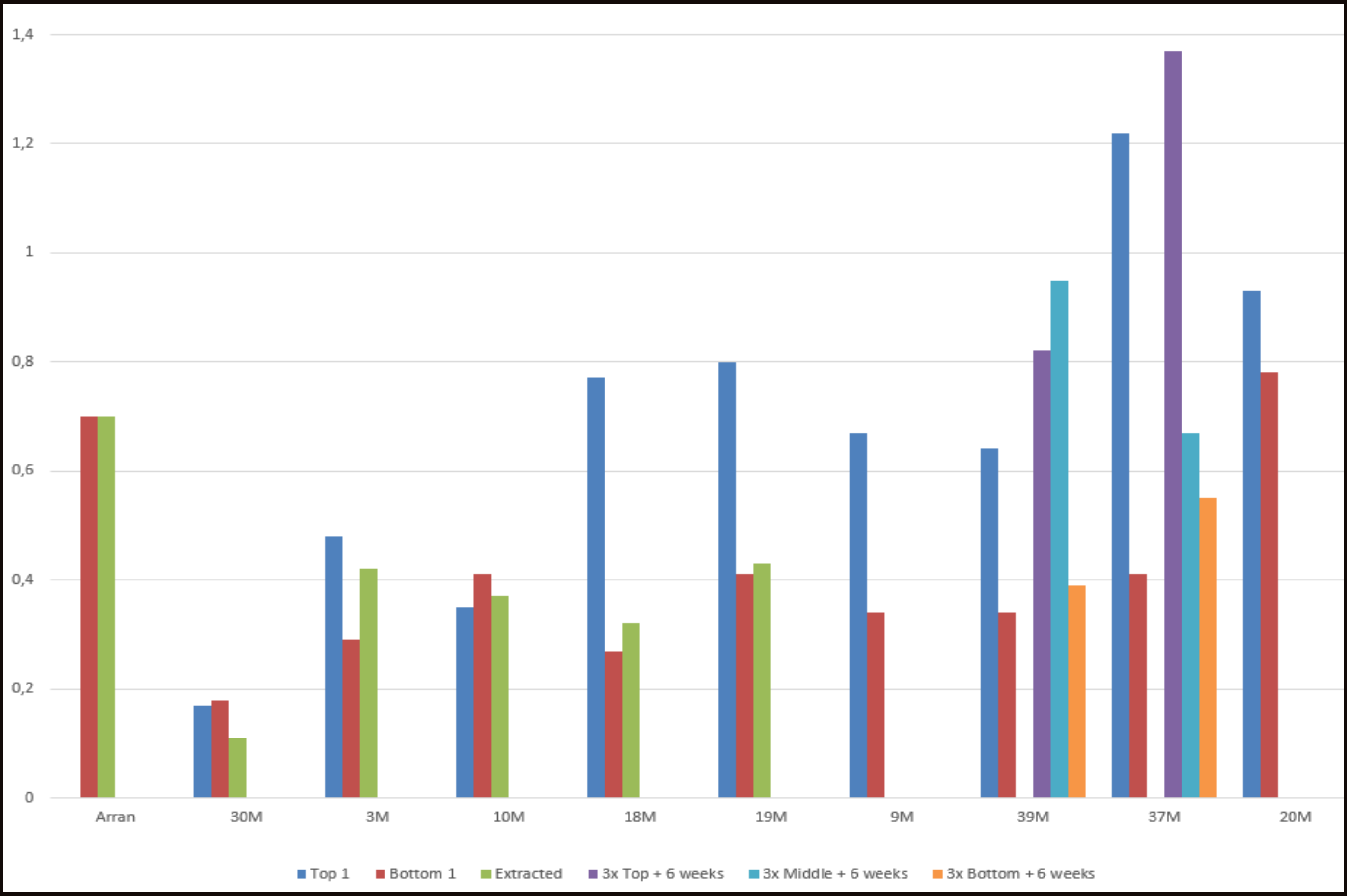
Another example is the quantification of M in crude extracts. I do this to see how much M is recovered before doing acetone-washings or recrystallizations. To prevent inevitable losses, people might consider to consume these whole extracts. I found them to conain 57-74% M (6 specimens).


M 0.5 - Sanchez 1mg per ml 0.5 AB M3 M10 M30.jpg
1758x657 pixels; RGB; 4.4MB
Results
File
Edit
Font
Results

	Area
1	39767.714
2	37110.191
3	38348.664
4	34907.170
5	30103.848

I dilute the crude extracts to 1mg/ml. This way, they can be nicely compared to a 2,5µg M-SO4 94% reference (above left).

Finally, I present the preliminary results of comparative TLC-value vs extracted M experiments.



What do you see in the graph? At the far left you have the ‘Arran’ column Results from this specimen were obtained from three punch biopsies that were taken 2cm apart in the bottom third of a bridgesoid. At the moment of sampling, I was not yet aware of the vertical M-gradient that seems te be present in these columnar cacti. Anyway, I used this cutting as a standard to predict M-contents of other cacti. Following the DCMQ-protocol as outlined above, this specimen got a 1.56 when compared to a spot of 2,5mcg M-SO4 94% and proved to contain 0,70% of extractable M-SO4 from dry whole cactus. Following samples were correlated with these findings, so that the relative intensities of their spots compared to the reference needed only to be multiplied by 0.4375 to obtain the predicted amount of M. In blue, you see these predictions originating from top samples (taken at +/- 1/10th of the total length. In red, you see the predicted M-content of a sample taken at 1/10th of the length from the bottom. It seems that for cuttings up to about 40cm (30M, 3M and 10M), the results are comparable. However, for the tallest specimens (50+ cm: ‘Arran’=the only bridgesoid between pachanois, 18M, 19M, 9M, 37M, 39M and 20M), a rather large difference was observed between the top and bottom samples. Preliminary findings show that the amount of extracted and recrystallized M is (unfortunately) best predicted by the bottom sample. Wanting to confirm this unexpected discovery of a M-gradient, I took samples from cuttings 37M and 39M at the same places, but now combined three ‘punches’ at each site and in the middle as well. You can see that the results nicely compare to the older ones. Indeed, I took these samples 6 weeks after the first and it seems there might be some increase in M-content (although it cannot be ruled out there are other explanations for this: drying, displacement,…?). The ‘middle’ samples were taken right between top and bottom samples, and show an inconsistent concentration. Perhaps the gradient is non-linear? Perhaps it has got something to do with older and newer tissue, or with sun exposure? So many things to examine further….

Of these specimens, I hereby also provide the TLC-calculated M-content of the crude extracts (as outlined above):

Arran : 70%

3M : 72%

10M : 66%

30M : 57%

18M : 74%

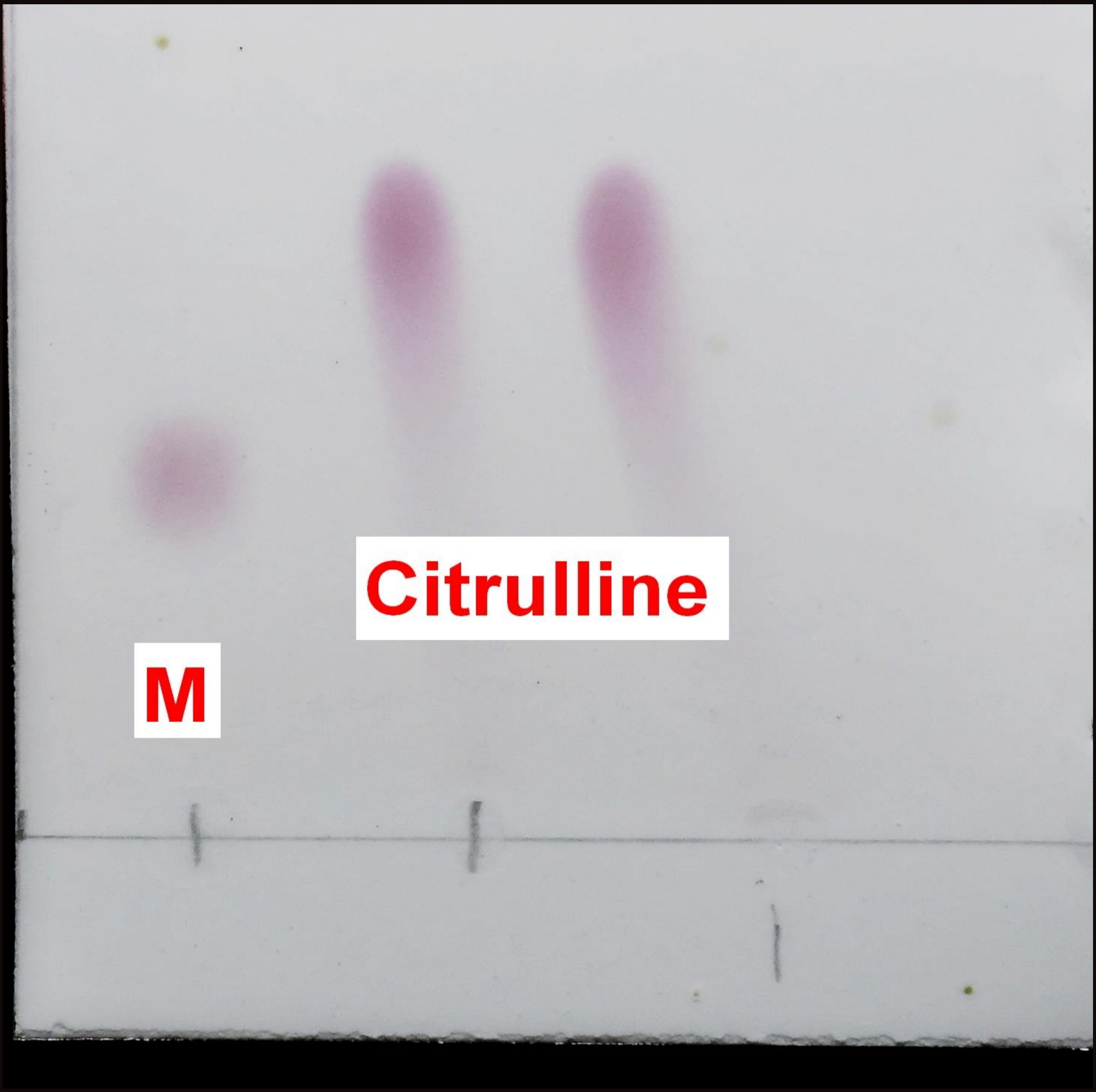
19M : 72%

In fact, the results under the heading ‘Extracted’(green) are made upon these TLC-calculations from crude extract. I purified and recrystallized three of them and found that I could indeed recrystallize about 90-93% of calculated M-SO4. This technique thus seems quite precise.

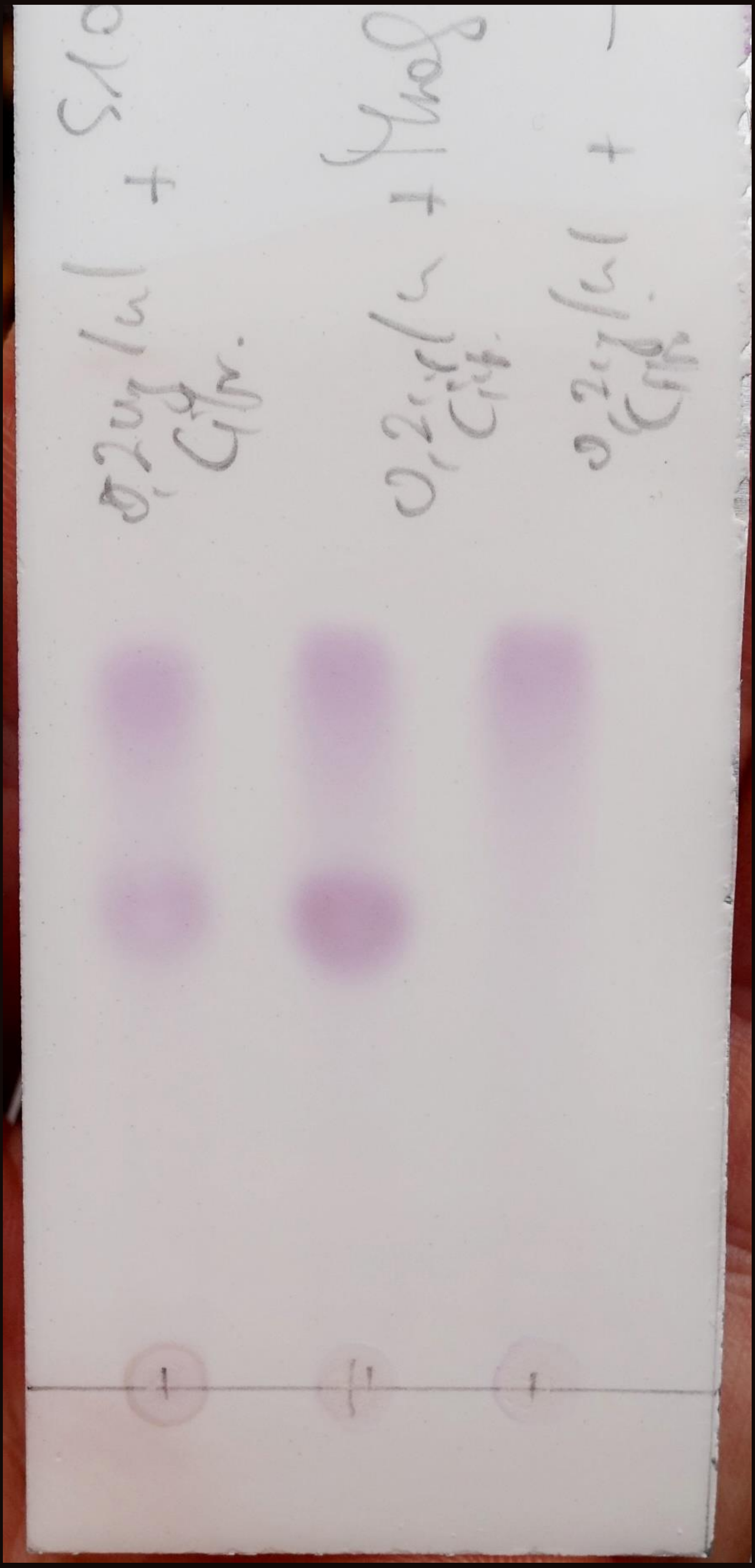
Keep in mind that these results are very preliminary and more data is essential to confirm the validity of the technique. But for now, the least we can say is that it seems worthwhile to dig deeper….

Another thing I thought about recently. Not everybody has some pure M-reference lying around. So at the Nexus, they propose to use caffeine as a reference for TLC. That seemed like a good idea, however ninhydrin doesn’t react with it and its Rf is a long way from M’s. So I looked around a bit and stumbled upon L-citrulline. This amino-acid is worldwide available in its pure form as a malate. It’s sold as a food supplement. I spotted some next to a M reference and was happy to see that it reacted with ninhydrin and that its Rf is quite close to that of M. Unfortunately, I don’t have the opportunity anymore to standardize this technique, but from this sole plate, I would say that a concentration of 0,2mg/ml might be a good starting point for experimentation. If someone could standardize this procedure, this would provide the community with a worldwide reference standard for the labelling of cactus clones. For example, in my current setup, I know that a spot with an intensity equal to 2,5µg of M-SO4 predicts an extractable M-content of 0,45% dry whole cactus.

It’s clear from this test-plate (that was damaged at the sides hence the displaced lanes) that 3,33µl of 1mg/ml citrulline malate is too concentrated. If diluted, the streaks will supposedly vanish. In fact, perhaps it’s possible to spot the citrulline on top of the test-samples, putting a reference above each sample?! So many questions still waiting to be answered…………….



I couldn’t help it and spotted another citrulline plate:



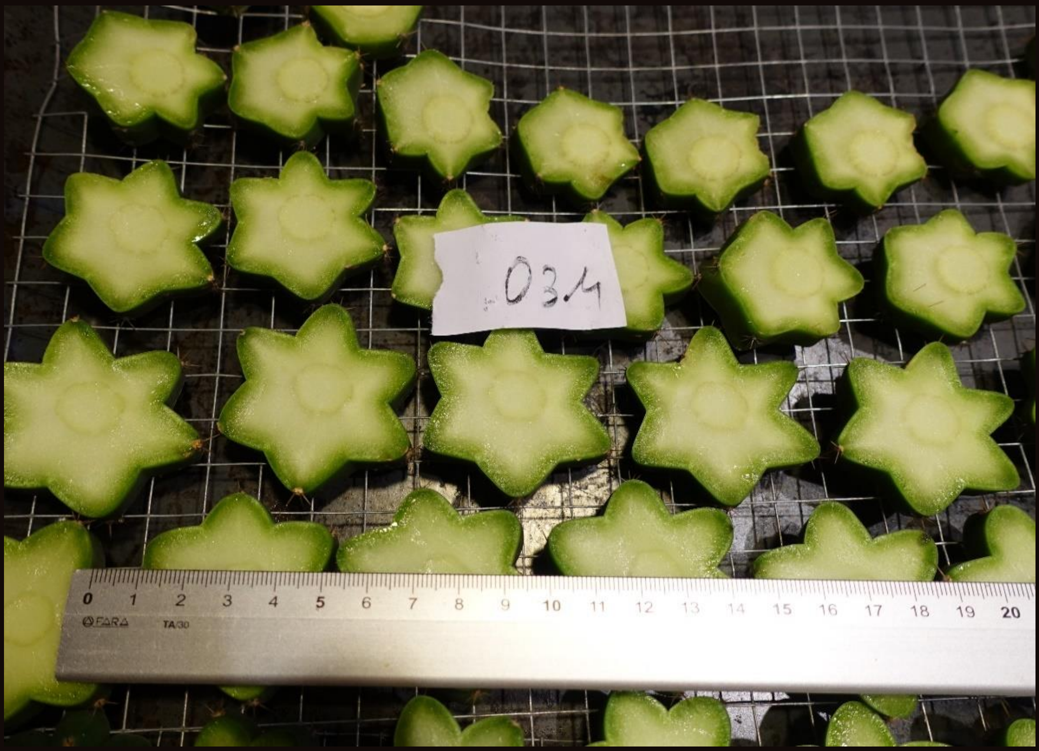
From left to right was first spotted: 6,66µl of sample S105 (classic DCMQ-procedure on pachanoi), next to it 2,5 µg of M-SO4 94% and the right lane was left unspotted. Then on top of these three locations, there was spotted 6,66µl of L-citrulline malate 0,2mg/ml. It can be observed that, nothwithstanding dilution, the streak-formation is still present and interferes with the M-spots. As well, the left citrulline-spot has a significantly lower ninhydrin-intensity than the others (as measured in Fiji), so perhaps it is delayed by the spotted plant-material?

Anyway, if citrulline doesn’t prove to be a good reference-candidate, there will surely be another readily available amine for standardization.

Keep up the good work, you researchers!

Extraction and purification notes

1) Optional: one or more punch-biopsies are taken from selected areas in the cuttings for research purposes.
2) The cuttings are weighed and cut into stars approx. 0,5 cm thick. Only the largest spines are removed. The skin and core are kept. If quantification is not an issue, the core may be removed for it should contain only a minimal amount of alkaloid and takes up considerable space in the Soxhlet-extractor. This would also speed up the drying process.



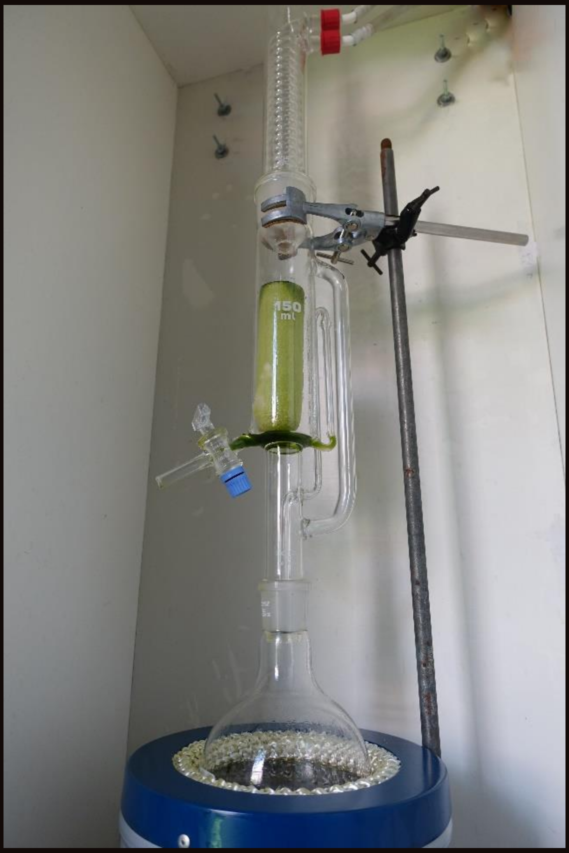
3) Now the stars are dried in a food dehydrator set at 50°C until cracker-dry.

4) In a coffee grinder, the stars are ground to a coarse powder. In contrast to cold-maceration where particle size must be as small as possible, too fine a powder will cause filter-jamming, caking and incomplete Soxhlet-extraction.

5) The powder is weighed and loaded into a thimble. Commercial ones are expensive and quite thick. In my experience, a thimble made from regular coffee-filters stapled or tied together allows for faster draining, better solvent-exchange and allows for a larger sample-volume to be extracted. I use a Soxhlet-extractor with a volume of 150ml combined with a flat-bottom flask of 500ml and a Dimroth-cooler connected to a garden pump that circulates cold water from a bucket that is cooled on regular (4-hourly) intervals with ice-packs. I strongly advise buying an extractor with faucet as this will make the following steps considerably easier.

6) The thimble is put in the extraction chamber and about 100ml of (ethanol >95%/ ammonia 24% 98/2) is added and left to sit for a few hours. This causes the alkaloids present in the sample to be converted to their freebase which increases their solubility in ethanol. Methanol could be used which might have better innate solvent properties for the salts, but due to its toxicity I choose not to use it. In the course of following extractions, I started to soak the thimble in the basic ethanol solution in a separate recipient and keep it at 60°C for 8+ hours. This converts the Soxhlet extraction to a Randall-extraction and allows for shorter Soxhlation-times. I do this by putting the recipient in a large volume of water, heat it to the required temperature, insulate it and let it cool overnight.

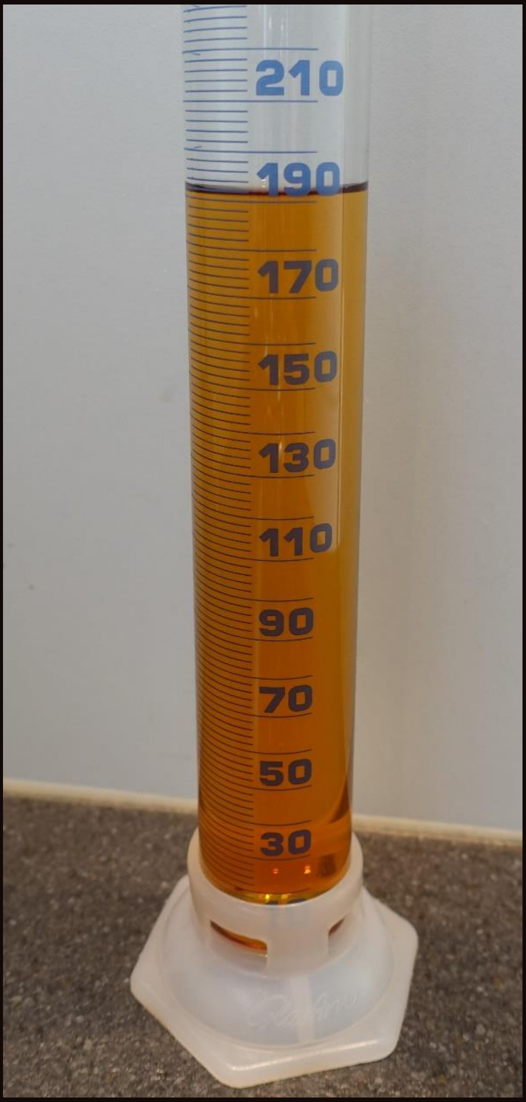
7) Now 200ml of non-basified ethanol is added to the flask and Soxhlation is started. Don’t forget to add boiling-stones! This setup is left to run for 8-24 hours (50-150 cycles), depending on the required degree of extraction. I TLC-tested the extract coming off after the seventh hour of soxhlation.It showed to contain about 0,6mg /cycle. I usually stop extracting after 8 hours (with Randall-soaking beforehand). If a large amount of material is to be processed, other thimbles can be hot-swapped in the course of the extraction.



8) Following this, the amount of extract is measured and distilled off until only 50ml remain (now the faucet comes in very handy). This way, about 200ml of ethanol can be recycled.

9) Next 200ml of (water/HCl 23% 98/2) are added which causes black/brown precipitation in the flask. A few more boiling stones are added (it seems the others are made ineffective by this step, possibly by debris clinging to them) and 100ml are distilled off, effectively removing most of the ethanol. This distillate could be fractionally distilled later on to salvage the ethanol.

10) Now the acidic solution is filtered to remove any precipitate. I use an improvised vacuum-setup with a wine-saver pump.

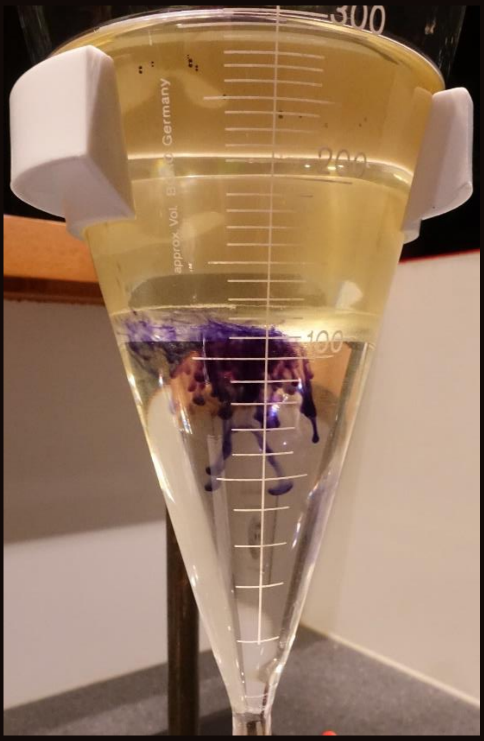


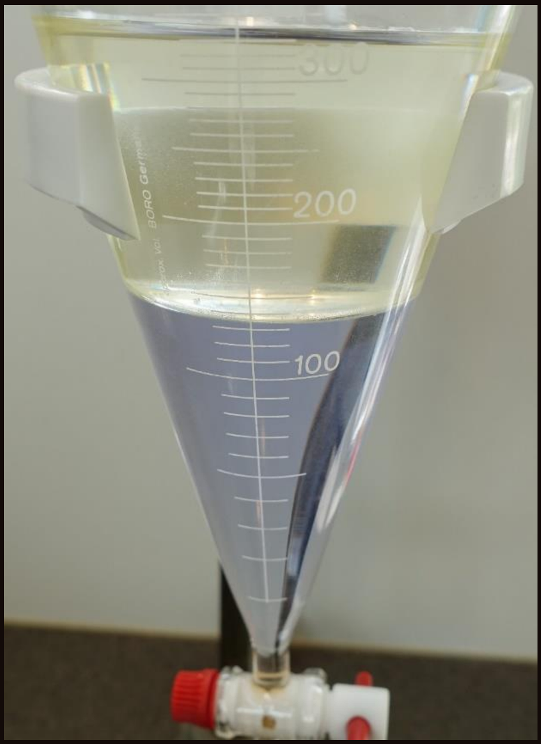
11) This dark-brown solution is added to a Squibb separatory funnel of 500ml and enough NaOH solution (50%) is added to obtain a total NaOH concentration of about 10%. Any cloudiness that remained in the solution will now disappear. In fact, I expected the contrary, but apparently, the solubility of the freebase alkaloids in water is enough to prevent their precipitation.

12) 70ml of xylene (or toluene) are added, and the separatory funnel is shaken vigorously for 3 minutes. Please check out some youtube-clips explaining the correct and safe use of this simple but very effective device. Gentle shaking will not allow for complete extraction. I tested this with a pure M-SO4 solution test-run. The funnel is left to stand and after a few minutes, a clear and complete separation is obtained. No emulsion-problems are encountered contrary to the water-based extractions I have used in the past. This makes the process much more enjoyable. The aqueous layer is drained, making sure no basic water is left in the apolar phase. It's better to have some apolar solvent in your aqueous phase at this moment.

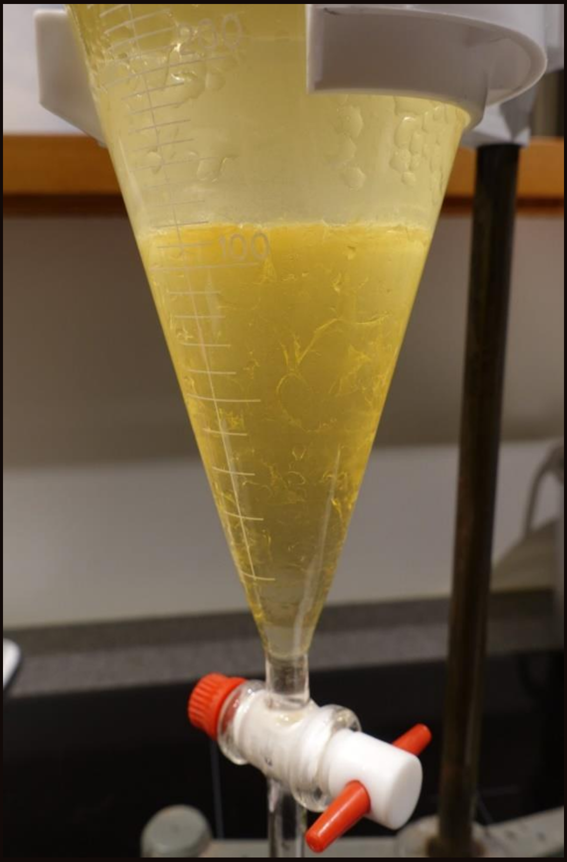
13) The aqueous solution is extracted another 2 times with 70ml of apolar solvent. Although not environmentally-friendly, the remaining basic solution can in fact be used as a highly effective drain-cleaner or it can be neutralized with HCl (caution!) and safely discarded.

14) The apolar extracts are now combined in the separatory funnel, and 100ml of demineralised water are added. Next, 0,2ml of 0,2% nitrazine solution are added to the funnel. The indicator will only mix with the aqueous layer, turning it just slightly purple-blue. I have found this method to be very convenient in the next step, but alternative ways of checking the pH are just as good.



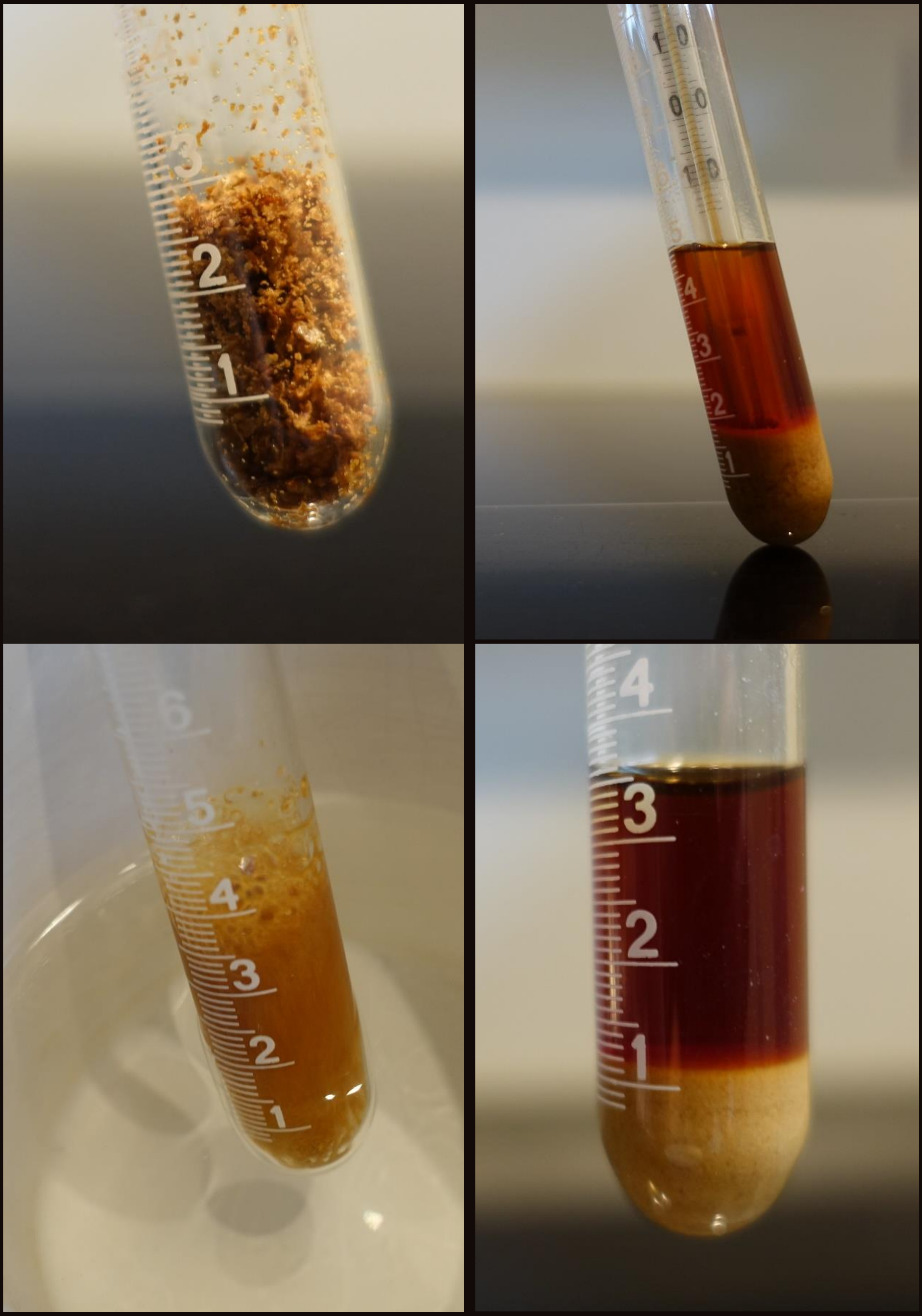


15) Now I add a H2SO4- solution of 5% 0,1ml at a time and shake vigourously in between. At a certain point, the aqueous layer will stay yellow even after shaking. At this point, some ‘floating veils’ will appear in the aqueous solution that can indicate complete titration in case no other indicator is used. The amount of H2SO4 -solution added already gives a very good indication of the amount of crude alkaloid that will be recovered.

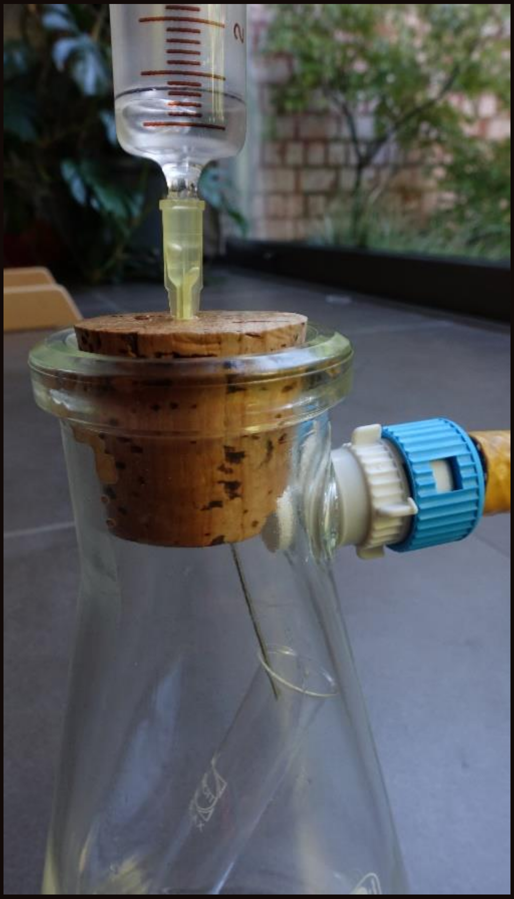


16) The aqueous layer is drained and left to evaporate in a PTFE-coated recipient over a steam bath. The crude extract is scraped up and weighed. The apolar solvent can be reused without further processing. Before I went to extracting cactus, I first ‘rehearsed’ the complete liquid-liquid extraction. I Was glad I did this, because it showed me the importance of really shaking the separatory funnel and not merely ‘swirl’ it with gentle shaking. Don’t hesitate to continue shaking the hell out of the mixture for about three minutes. You don’t get annoying emulsions after an alcoholic extraction, so there’s no reason to save that elbow grease 😊.
    I did a ‘clean run’ of the steps with 400mg M-SO4 94% dissolved in 300ml of demineralized water to which 1ml of HCl 23% was added to mimick the acidic conditions of the real ‘soup’. After extracting the solution following the above steps, 375mg of M-SO4 were recovered (=yield 94%). So you can be pretty sure there’s not much loss here. Some people would stop here, ingesting the crude extract. If they should choose to do so, I strongly advise to do a TLC-comparison of extracts at 1mg/ml to a known standard. This procedure is outlined above and will tell them the M-content+ of their extracts, give or take a few percent.

17) The crude extract is then washed with anhydrous acetone (dried with MgSO4). At the moment, this step is the most ‘lossy’ in the whole prufication-process. In a ‘clean run’ with pure dissolved M-SO4, losses after washing were 10%. Instead of the technique described below, I would now do many room-temperature/warm acetone washings in a mortar and pestle, each time waiting some minutes to let the fine mist of particles settle before decanting. I would omit the freezing step after each washing because of condensation-problems in a water-free environment. When the washing liquid comes off sufficiently clear, all washings are collected together and they are put at -20°C for 8 hours. The recrystallized material is collected, dried and added to the washed extract. This alternative technique is to be tested and its losses to be calculated in the future.
    Now for the technique I used up until now. Small quantities are crushed within a test tube, larger quantities are ground with mortar and pestle within the anhydrous acetone. I tried skipping this step and going directly for a recrystallization, but what remains after the acetone has evaporated (a black oil) has made me change my mind. I wash the extract 3-4 times with a small quantity of acetone, bringing it to a boil in a hot water bath before chilling the recipient again and removing the solvent. This step removes a lot of junk from the crude extract. In my experimentation, the crude extract I got from Trich Bridgesii and Trich Pachanoi using the above techniques showed to contain about 60-75% M. So in this step, quite some weight will be lost. I try to keep M-losses to a minimum by using dried and chilled acetone, but nevertheless there will be some loss of yield. One day I will spot the dried acetone-washes to get an idea of the exact amounts we’re talking about. You can also use a small-scale filtration setup to salvage floating crystals from the washing liquid (see below). Anyway, purification inevitably leads to losses, but that’s the price you pay for a crystal pure experience.

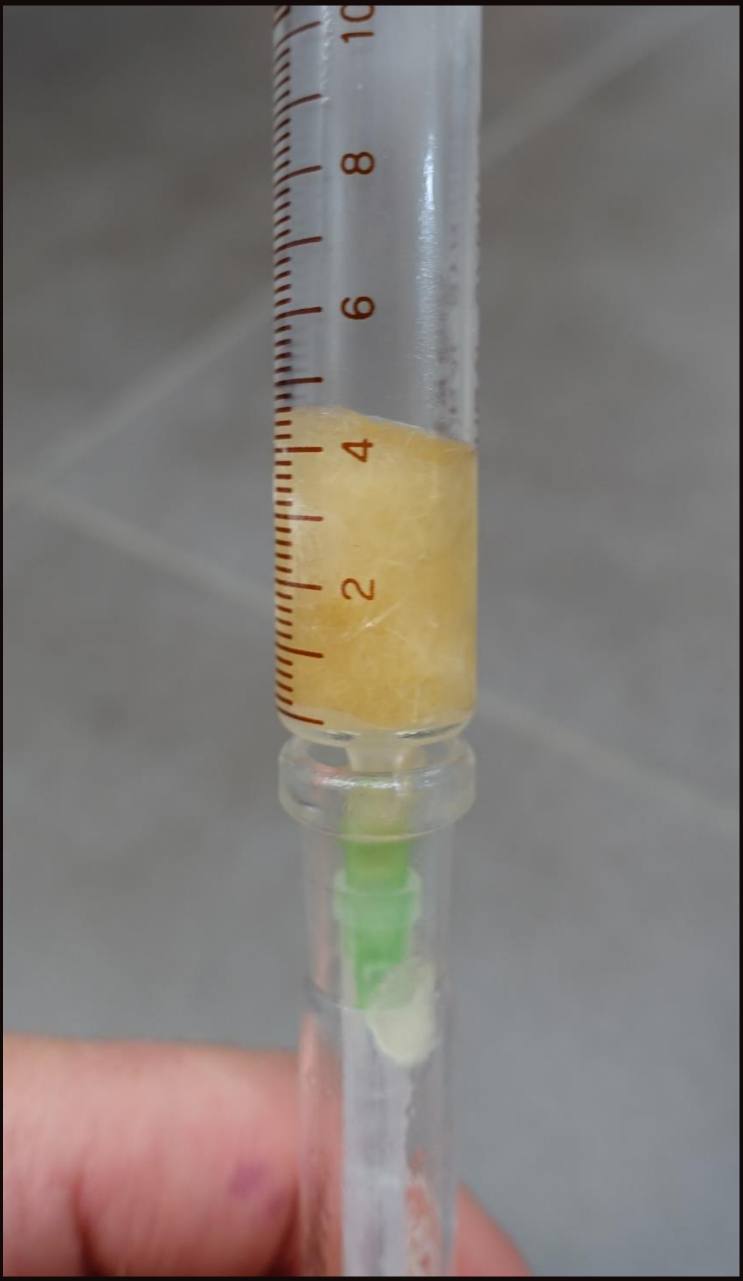


18) Optionally, now one does an activated charcoal purification by dissolving 1000mg in 9ml of demineralized water, heating it in a waterbath until all crystals are dissolved, adding 9ml of acetone and 20mg of activated charcoal. A small boiling stone is added and the solution is boiled for a few minutes. At about 60°C, acetone will boil out of solution providing the constant mixing that facilitates tha activity of the charcoal. Now the solution is filtered through a hot glass syringe in which a small circle of fine filter-paper is put in the bottom. The solution is poured in, and the plunger is pressed down, forcing the liquid through the filter paper into another recipient for recrystallization. Alternatively, one can use a vacuum-setup where a long needle put on the syringe is forced through a stopper on a vacuum-flask. Within the flask, a long tall recipient (test tube) rests in the neck of it and the needle protrudes into the tube. This way, one has created a micro-Büchner vacuum filtration setup.



19) Now for the recrystallization itself. This step further purifies your crude extract to 90+%. In a ‘clean run’ with pure M-SO4, a two-step recrystallization recovered 92% of material. In a TLC-controlled recrystallization of crude extract, the yield of recovered alkaloid from its total in the extract was 91% after two steps. ‘Two-step’ means that the mother liquor from the first recrystallization is evaporated to dryness, weighed and redissolved in a corresponding amount of solvent. It’s up to you to decide if a third step is worth the effort, depending on the amount of material you’re working with. I did some experimentation, and came to the following rule of thumb: in a hot water bath of about 50°C, every 1000mg of cleaned M-SO4 is dissolved in 9ml of demineralized water, then 13,5ml acetone are added under stirring and then dropwise until the cloudiness won’t disappear. The solution is warmed in a hot water bath of about 80°C and is left to boil until the solution turns clear again (adding a boiling stone promotes safety). A stopper is placed on the recipient which is left to cool slowly to room temperature. In my experience, crystals don’t always form spontaneously and I have to initiate crystallization by putting the recipient in the freezer for a few minutes. Then I take it out and let it crystallize further in the fridge. This way, about 65-75% of M-SO4 can be recovered. The mother liquor is removed, dried and the process is repeated. Three steps recover about 95% of M present.

While devising the micro-vacuumfiltration setup, I thought about a more direct way with less steps and thus less lossy. Why not recrystallize in the syringe itself and use the filter at the bottom as a Büchner? It works! After chilling, the plunger is pressed down, effectively filtering the mother liquor into a waiting test-tube or on a no-stick heatable surface for drying, weighing and recrystallizing. Then the plunger is used to compact the crystals in a fine or coarse ‘net’ or ‘fabric’, depending upon the size of the crystals. A small volume of cold acetone is then poured in the syringe and is squeezed out again into the test-tube. This is repeated another time. Now the syringe is lightly warmed and after all the acetone has evaporated, the plunger is inserted, the syringe is kept upright and a needle is inserted into the syringe so that it keeps the filter stuck to the plunger while the latter is slowly pulled out. The net is easily seprated from the filter that can be reused for the next recrystallization. In the pictures you can observe some active charcoal on the filter that was left before recrystallizing. This has proven to be an effective and low-loss technique. I’ve ordered a glass syringe of 30ml now for larger batches.



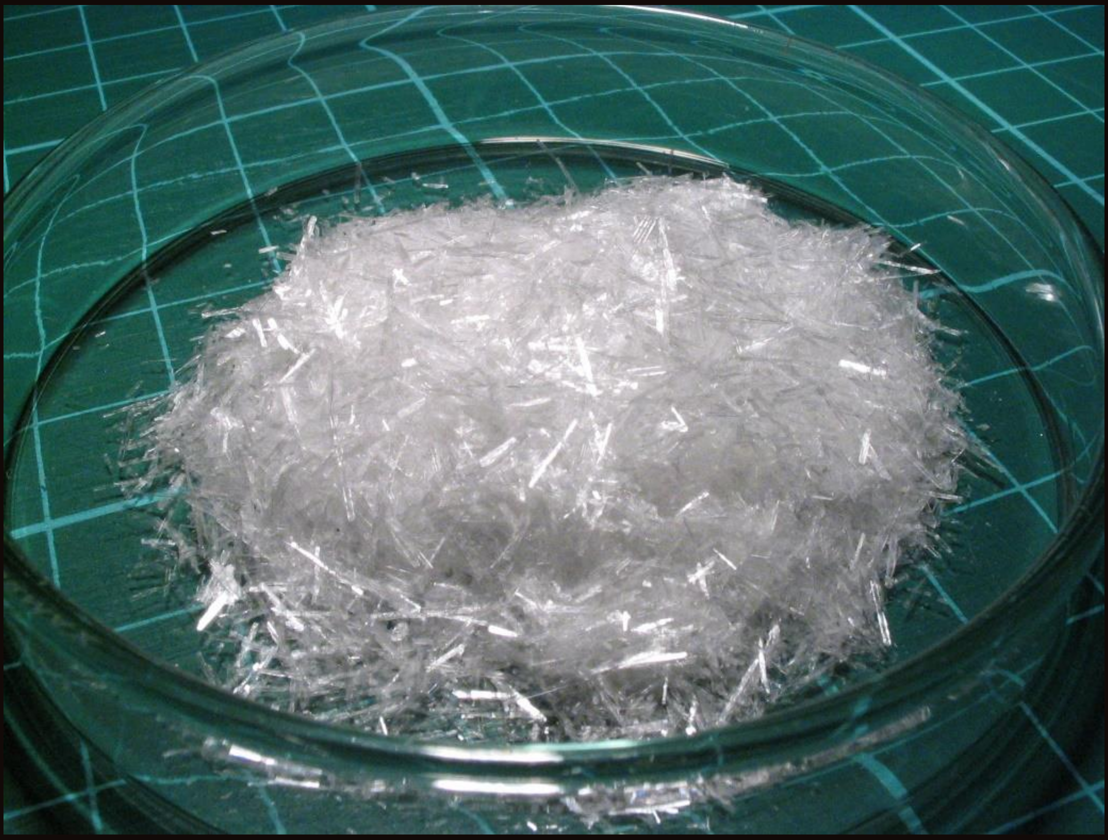
2
4
6
8
10ml

2
4
6
8

M-SO4 recrystallized twice in this manner looks like this:

You're looking at a 'museum dose' of M-SO4 of about 350mg. When left to slowly crystallize from water, the above crystals form beautiful plates:

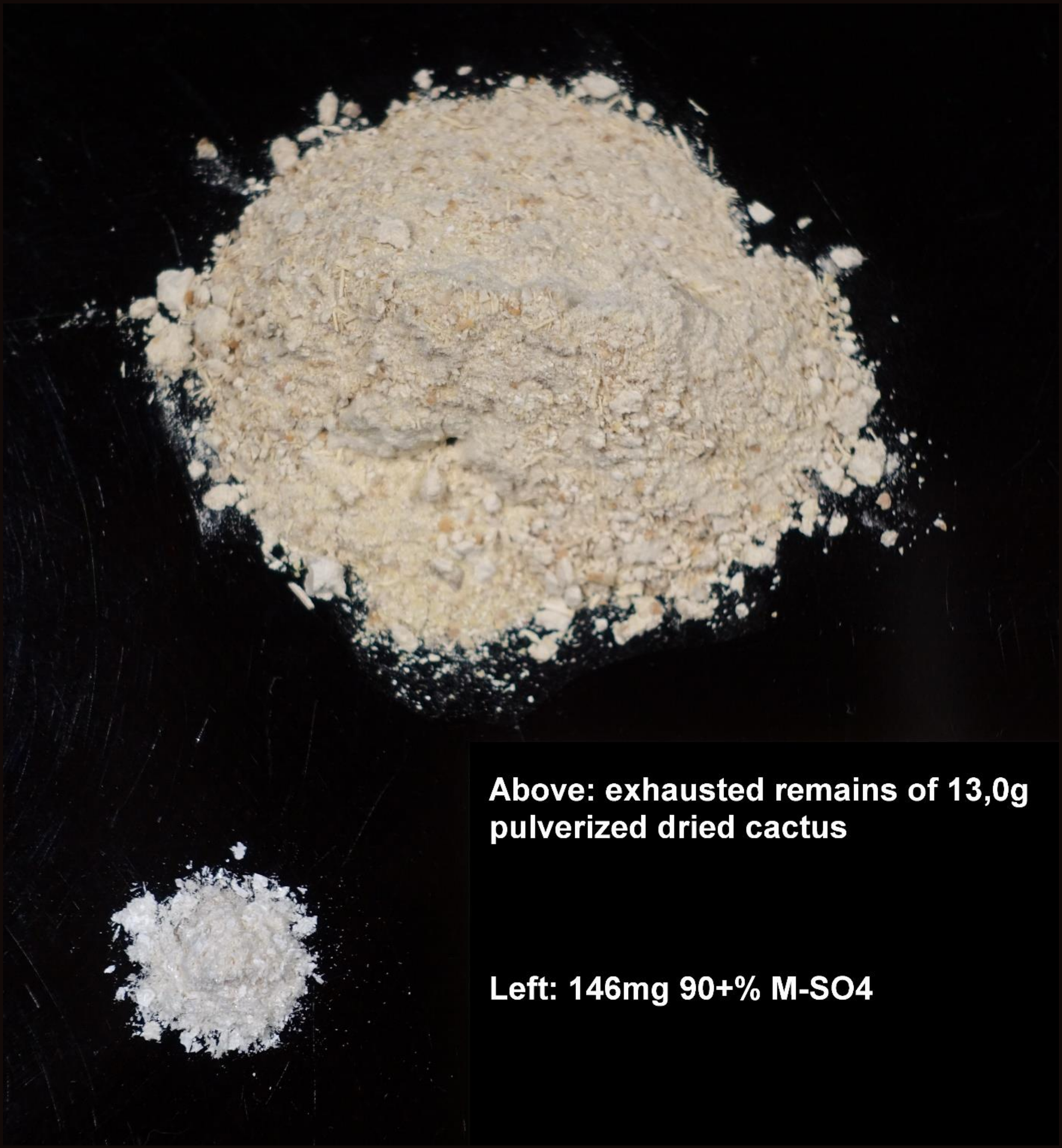
When recrystallized three consecutive times, M-SO4 looks like this (the grid is 1x1 cm):



This is the M-SO4 that I got from a much earlier STB-extraction and that was recently sent to Energy Control for analysis. It proved to be 94% pure. In fact, this sample was extracted 11 years ago and was stored in a dark recipient in the attic where temperatures varied tens of degrees. Talking about shelf life! This is the reference material used for TLC-analysis.

So there you have it: we went all the way from plant to crystal. The picture below shows the exhausted cactus fibres and the magic it contained (Trich bridgesii 'Arran', 13g was the starting dry weight).

Good luck and safe journeys!



Above: exhausted remains of 13,0g pulverized dried cactus

Left: 146mg 90+% M-SO4